

Identifying migraine genes

Finemapping a Previously Discovered Migraine Locus on Chromosome 10q22

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Biology

Abstract

Migraine is a common hereditary disorder affecting 10-12 % of the adult population. Previous studies have shown that migraine is caused by hereditary complex characters, meaning that migraine is not caused by a single gene, but by a combination of genetic and environmental factors. As migraine is inherited in a non-mendelian fashion, so called end diagnosis based linkage studies of migraine have provided poor results.

A recently introduced trait based system of phenotype creation was used in this study. The study sample of 438 individuals (252 females and 185 males) was genotyped with 35 microsatellite markers. Before genotyping the data was prepared by examining the successfulness of the electrophoresis and PCR reactions. The data was manually inspected, by the author of this report, for color bleed, absolute and relative product amounts and the shape and size of the standard peaks. The success rate of the markers (90.6 %) was close to the expected value, even though one marker had to be completely rejected from the study.

Allele peaks were evaluated based on, shape, relative and absolute size and correspondence to their expected locations. Data obtained from the genotyping was inserted into a statistical analysis program *LINKAGE* in addition to several other programs. Linkage and association analyses were performed on the data obtained from genotyping, together with the corresponding pedigree data and respective p- and LOD values were obtained for all markers.

The linkage analyses allowed us to define the newly discovered, 10q22, migraine locus with a high level of detail, and to accurately define the area of interest for further studies. These results were observed for several markers and phenotypes with sufficient evidence of a linkage signal to this locus, and allowed us to determine several positional candidate genes. The *KCNMA1* gene, a calcium channel in the brain, and *NRG3* gene, involved in regulating the neural network, are located close to the markers in question. *KCNMA1* or *NRG3* have not been previously being linked to migraine. Markers studied on other chromosomes showed only nominal evidence of linkage. Some problems associated with the genotyping progress were identified to possibly result from incorrect binfiles and errors in the PCR process. The success of the genotyping process was satisfactory and in par with previous work. It is suggested that the migraine locus on 10q22 finemapped in this study may play an important role in migraine pathophysiology.

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1. Introduction

Migraine is a common episodic disorder affecting 10-12% of the adult population¹. The most common symptom of migraine is unilateral, pulsating headache. This is often accompanied with nausea and photophobia etc. Migraine can be classified with the International Headache Society (IHS) classification rules (Appendix 1) into several different subtypes that are often used in the diagnosis of the disorder.¹¹ The biological cause of migraine has not been fully determined, but it is believed to be linked to dysfunctional ion channels³⁻⁴.

Migraine has been identified to have a hereditary component in several studies.⁵ Several loci linked to common forms of migraine have been discovered. However, no genes affecting the susceptibility for the two most common types of migraine, migraine without aura (MO) and migraine with aura (MA), have been reported so far. Most efforts in mapping complex traits have used end diagnoses, conclusions based on the analysis of symptoms, as phenotypes. Anttila et al² however, suggested that this model is oversimplified and that mapping of the genetic factors of migraine based on trait component will be more effective than diagnosis based mapping; especially, because migraine diagnoses are mostly based on patient history². Trait component analysis is a novel approach in mapping migraine loci and its applicability has not yet being fully proven.

The absence or presence of Mendelian characters is determined by the genotype of a single locus. This does not mean that a single gene contributes the trait, but that together with the normal genetic and environmental background of the organism, a genotype at a single locus is sufficient and essential for the character to be expressed. Over 10000 such traits, both pathogenic and non-pathogenic, have been identified in humans to date. More difficult to identify have been traits that are not inherited in a purely Mendelian fashion. These traits are often prevalent in the same families from generation to generation. Their cause is partly genetic, arising from effects of genotypes in many loci, and partly environmental. Single genes contributing to such complex characters do not determine if a trait is expressed or not, but can rather affect how it is expressed or increase or decrease the probability of the phenotype being expressed. Mapping complex traits has proven to be difficult in large part due to the complications involved in determining what are the actual effects of individual genetic factors, as opposed to gene-gene interactions or environmental effects. In this regard it is somewhat

easier to locate severe pathogenic complex characters, because they exhibit clearly distinguishable clinical symptoms, usually allowing relatively easy classification and collection of large samples².

Generally the human genome is extremely similar between individuals, with differences occurring on average between every 1500 base pairs. Usually these differences are small, but in rare cases entire sequences can be missing or mutated. Mutations can take place during natural occurrences like cell division or be caused by external factors like radiation. These changes are mostly harmless, but in some cases they are pathogenic in nature. Genetic differences between individuals can also be used in trait mapping, because the human genome also includes numerous sequences of repeating di-, tri- and tetranucleotides. The numbers of repetitions of the sequence in question are useful in mapping differences between individuals, as millions of such microsatellite markers are known. Each marker has several alleles, which can be distinguished via electrophoresis. If two individuals share a copy of the same allele of the same marker they are also likely to share genetic material close to the marker. Linkage and association analysis of trait mapping is based on this property⁸.

Several previous studies in Finland and Australia have indicated possible migraine loci in chromosome 10q22². The purpose of this study is to genotype the previously identified loci with microsatellite markers and by analysing the data to determine candidate genes for further studies. Determining the genetic factors behind migraine is important so that the disorder can be physiologically understood, diagnosed, and treated. On a larger scale, developing complex disease mapping methods is vital in the search for genetic factors governing the human body.

This study was based on the hypothesis that a novel migraine-related region of the human genome, identified in a genome-wide scan, contains a gene affecting migraine susceptibility. This region was fine mapped with microsatellite markers and the genotyping results, together with family relationship and symptoms data of the individuals taking part in the study, were analysed with linkage and association analysis software. Evidence of linkage was observed on chromosome 10q22 for several markers giving more details of this recently discovered migraine locus.

2. Materials and methods

2.1 Study sample

The study sample consists of 438 individuals (252 female and 185 male) and was collected from 45 unrelated multigenerational Finnish migraine families. The families included in the study have been selected based on a high prevalence of migraine. The study sample was the same as used by Anttila et al.² in a genome-wide scan that located the migraine loci investigated in this study. Individuals were diagnosed in accordance with the (2004) IHS classification rules (Appendix 1)¹¹ and based on extensive validated questionnaires. The ethics committee of the Helsinki University Central Hospital approved the study protocols.

A total of 35 microsatellite markers in the chromosomes, 4, 10, 17 and 18 were investigated to finemap the previously identified migraine-linked regions. Chromosome 4 had 6 markers, chromosome 10 had 16 markers, chromosome 17 had 5 markers and chromosome 18 had 7 markers.

2.2 Detection of sequence variants

2.2.1 Sample preparation

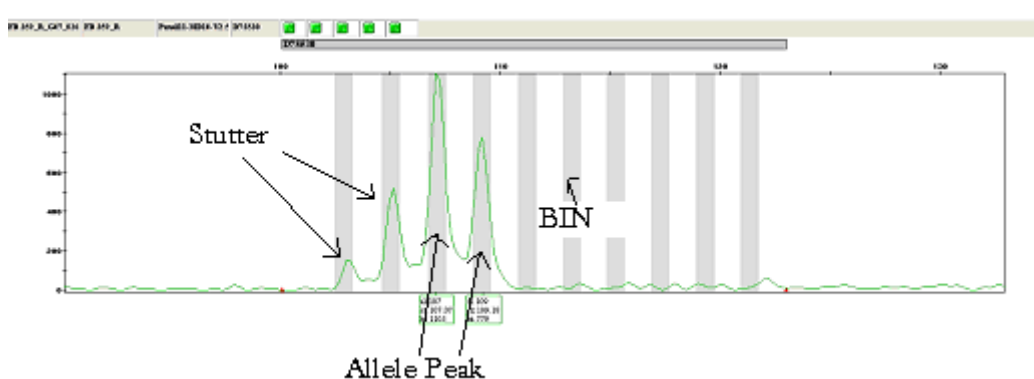
DNA fragments were separated with the ABI 3730 DNA Analyzing System by *Applied Biosystems*. The fragment analysis data was analysed semi-automatically with the *GeneMapper* program by *Applied Biosystems*. The samples were analysed in panels containing 96 samples, including controls. Each panel contained designated markers for the set. Before genotyping could be done from the fragment analysis data, several properties of the data had to be manually analysed. First of these was the manual inspection of the height and shape of the size standards. For this study acceptable peaks had heights between 1000 and 3000 relative fluorescence units (rfu), were slim and single peaked. The peaks were also expected to be similarly sized and have no extra peaks between the standards. This analysis was performed with the *GeneMapper* software. Possible shortcomings to these criteria were taken note of in accordance with the general performance policy of the Finnish Genome Center.⁹ The genotyping process was done by the author of this report.

Product amounts were also manually compared against the amounts of the standards. This was done by comparing the heights of the standard peaks to the heights of the product peaks. The product peaks were expected to be higher than the standard peaks and to be between 1000 and 5000 rfu in height. Water samples were expected to contain no product. Products of all dyes were inspected from the same graph. Color bleed, incorrect mixing of different dyes, was also checked for. Possible shortcomings to these criteria were taken note of in accordance with the general performance policy of the Finnish Genome Center.⁹

2.2.2 Genotyping

After the data had been prepared for genotyping the *GeneMapper* program by *Applied Biosystems* was used to automatically detect alleles. Each sample had several markers that were searched for. If an individual is homozygous, having inherited the same allele from both parents, a single allele peak can be observed from the graph produced by the analysis program. However, if the individual is a heterozygote, having inherited different alleles from her parents, two allele peaks can be observed. Each peak corresponds to a specific allele of the marker in question. This link allows the detection of sequence variants, as individuals observed with different alleles are also likely have different genetic material around the marker. The markers used in this study were polymorphic microsatellite markers based on repeating di-, tri- and tetranucleotides. The number of these repetitions varies between different individuals, with numerous known mutations in the human population.

Figure 1. *GeneMapper* allele identification graph



The allele identification process described below is depicted in Figure 1. The graphs are read so that the correct peaks are aligned with the so-called “binfiles”, visible as horizontal grey bands in Figure 1, that contain the expected locations of all the known human variations of the marker. The automatic genotyping program does not, however, take the possible variations into account; meaning that it may falsely identify a peak outside the known variations. Correct allele peaks are generally symmetrical and evenly shaped, as seen in Figure 1. The genotyping program can identify clear allele peaks if no interference has occurred in the electrophoresis process. However, the program often mislabels peaks in more complex cases, thus causing a need for a manual inspection of all the graphs.

Table 1. Allele peak acceptance criteria

Peak property	Acceptance criteria
Height	Above 100 rfu
Shape	Slim and symmetrical
Location	On a binfile
Number	One or two per sample
Relative sizes	Not more than 2 times higher than other peaks

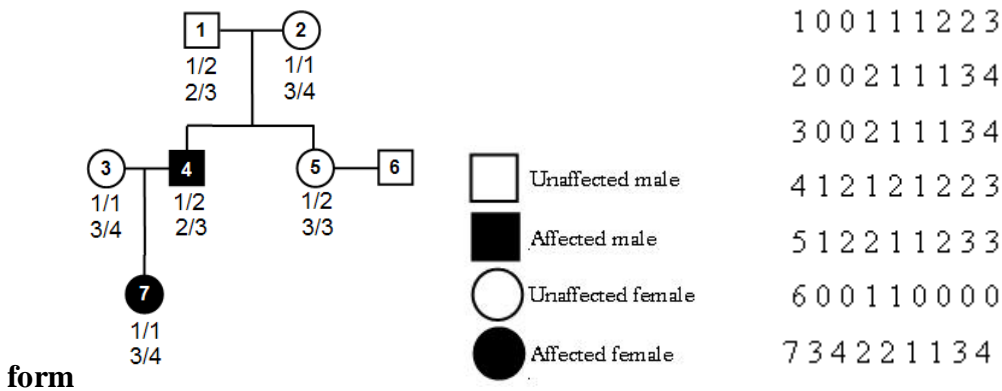
Due to the strict inclusion criteria in the genotyping process numerous allele peaks have to be rejected. If an allele peak was rejected from an individual then the whole marker for the individual in question was rejected. Only allele peaks with heights 100 rfu and above were used in the study, as smaller peaks are prone to too much interference. Another problem with peaks sizes is that the analysis program usually identifies the tallest peaks as the allele peaks. This may lead to stutter or constant peaks to be falsely identified by the analysis program as allele peaks. In these cases the researcher had to manually identify the correct peaks. Great height difference in peaks (the other peak being over 2 times higher than the other) is also a reason for rejection. The acceptance criterion of the allele peaks are summarised in Table 1.

2.3 Linkage analysis

The data obtained from the genotyping was combined with family data collected from the sample donors by the Finnish Genome Center. The family data used in the study was arranged into pedigrees in a numerical form understandable by the analysis program (Figure 2). Phenotype data was also included in the family information. Phenotypes were formed by using the nine individual traits (aggravation by physical exercise, attack length, intensity of pain, nausea, phonophobia, photophobia, pulsation, unilaterality of the headache, and vomiting) and five trait groups (associated symptoms, IHS full criteria, nausea and/or vomiting, pain criteria, and photo- and phonophobia)¹¹, as described by Anttila et al.²

The method is based on the theory that the marker and trait loci are likely to be more common in the descendants of the individual with the original mutation, than in randomly selected individuals resulting in transmission disequilibrium of the mutation. This means that in an isolated population (such as Finns), the same genetic material close to the allele is more likely to be shared by most carriers of the allele, but in older populations, through recombinations, the genetic material around the mutation becomes varied across the population.

Figure 2. Sample Pedigree in family tree form and file



Linkage analysis was performed using the *Analyze* package.¹² Linkage analysis is used to locate certain trait causing genes in the genome. Linkage analysis is based on the maximum likelihood assumption that tests the so-called zero hypothesis (the allele has no linkage to the

wanted trait) to other hypothesis where the allele is linked to the trait in question. In essence the analysis program compares, which alleles are shared by individuals expressing the trait and which are shared by individuals not expressing the trait. Linkage analysis can be either two-point linkage analysis or multipoint linkage analysis. The difference between the two is that in two-point linkage analysis one marker at a time is used, while in multipoint linkage analysis several markers can be used. Linkage analysis can also be parametric or non-parametric, depending whether model of inheritance for the trait in question is specified. Both analyses and approaches were used in this study. The hypotheses are mathematically tested so that they produce a LOD (logarithm of odds) value. An LOD value of 3 or higher is usually considered to be statistically significant in genome-wide linkage analysis and is the equivalent of a p-value of 0.001. The boundary of significance is individually determined for each study and is dependant on the pedigree structure of the families involved in the study. Parametric and nonparametric LOD scores were calculated using an affecteds-only strategy (meaning that all individuals not scored as affected were considered as unknowns) for the 35 markers genotyped.

2.4 Association analysis

Association analysis was performed using the *Mendel* program.¹³ Association analysis aims to find if a defined property in an individual is more likely to be inherited with a certain allele. Association analysis works especially well with populations that have a small founder population with a mutation carrier and have quickly grown in size after the population bottleneck. This leads to many mutation carriers sharing the same genetic material around the mutation. In this study the association analysis was performed within families by comparing what alleles affected individuals share with each other, but not with the unaffected individuals. The validity of association is measured with the p-value. A limit for the statistical significance of the p-value is set individually for each experiment⁷. However, association analysis is not very reliable in locating complex characters, and thus rarely used, making it necessary to critically examine the obtained results.

3. Results

3.1 Marker acceptance rate

3.1.1 Sample preparation

Most samples were of good quality, although several different problems were noted with some samples. This did not, however, result into sample rejections. The heights of several standards were noted to be too short (below 1000 rfu) in several panels. Standard peaks were also of uneven heights (with at least one peak over two times higher than another peak). In some panels too little product (less than of the standard) was present. Color bleed was also observed in numerous panels. These observations were recorded into the Finnish Genome Center database for further study.

3.1.2 Genotyping

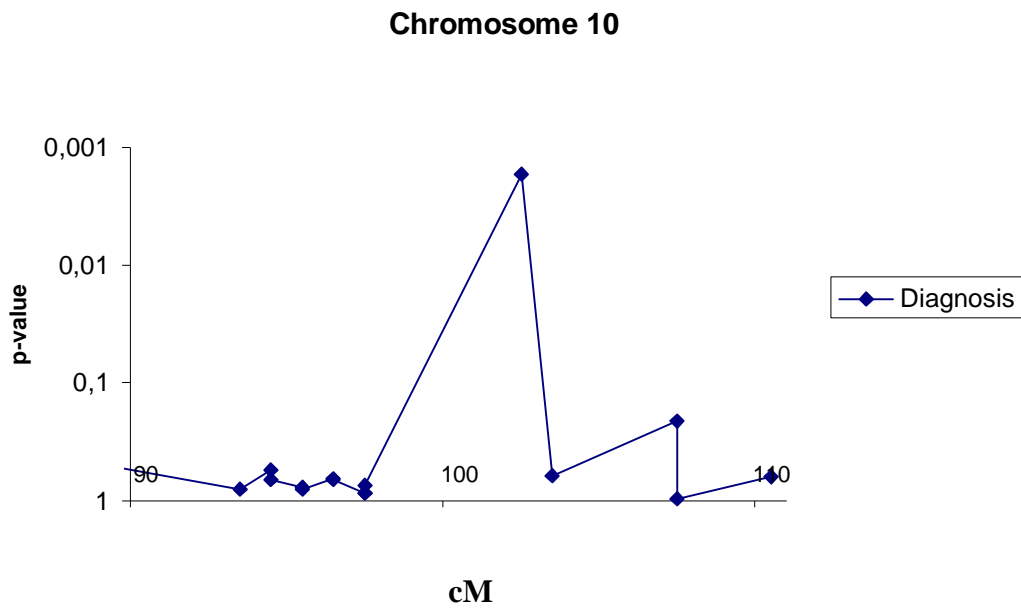
Table

Allele peaks corresponding to the allele peak acceptance criteria were accepted and marked by the genotyping program *GenoMapper*. Allele peaks that were of, incorrect height (below 100 rfu), incorrect shape, (not symmetrical and thin enough) and incorrect location were rejected. Some samples were also rejected, because one of the peaks was rejected or because no allele peak could be located in the electrophoresis graph. One entire marker was rejected from the data due to its inability to produce identifiable allele peaks. The analysis program also, incorrectly, identified some allele peaks in the water samples. A total of 90.6 % of samples were accepted and 9.4% rejected from the study.

3.2 Association analysis

The association analysis of the markers in chromosomes (4, 17 and 18) did not produce meaningful results. Association in Chromosome 10, however, was of a potentially interesting level for the diagnosis phenotype (Appendix 3). P-values against their respective markers are plotted in figure 3 in an inverse logarithmic (base 10) scale.

Figure 3. TDT association analysis



3.3 Linkage analysis

We were able to define the newly discovered migraine locus on chromosome 10q22 in greater detail. However, after my primary analysis the data was re-inspected by Anttila et al after a study in Australia, unpublished, showed suggestive evidence of linkage at the locus in question. Several corrections were made to the family data. After these procedures the data was reanalyzed with the author of this report taking part in the process. Both multipoint (Figure 4) and two-point linkage analyses (Figure 5) of the revised data showed suggestive evidence of linkage. The highest linkage scores (multipoint NPL LOD: 2.39 two-point HLOD: 2.14) were observed at 104 centimorgans (cM), in chromosome 10q22, with several nearby markers also showing suggestive evidence of linkage. Two genes, KCNMA1 and NRG3, were noted to be situated close to the area.

Figure 4. Chromosome 10 Non-Parametric Linkage Analysis

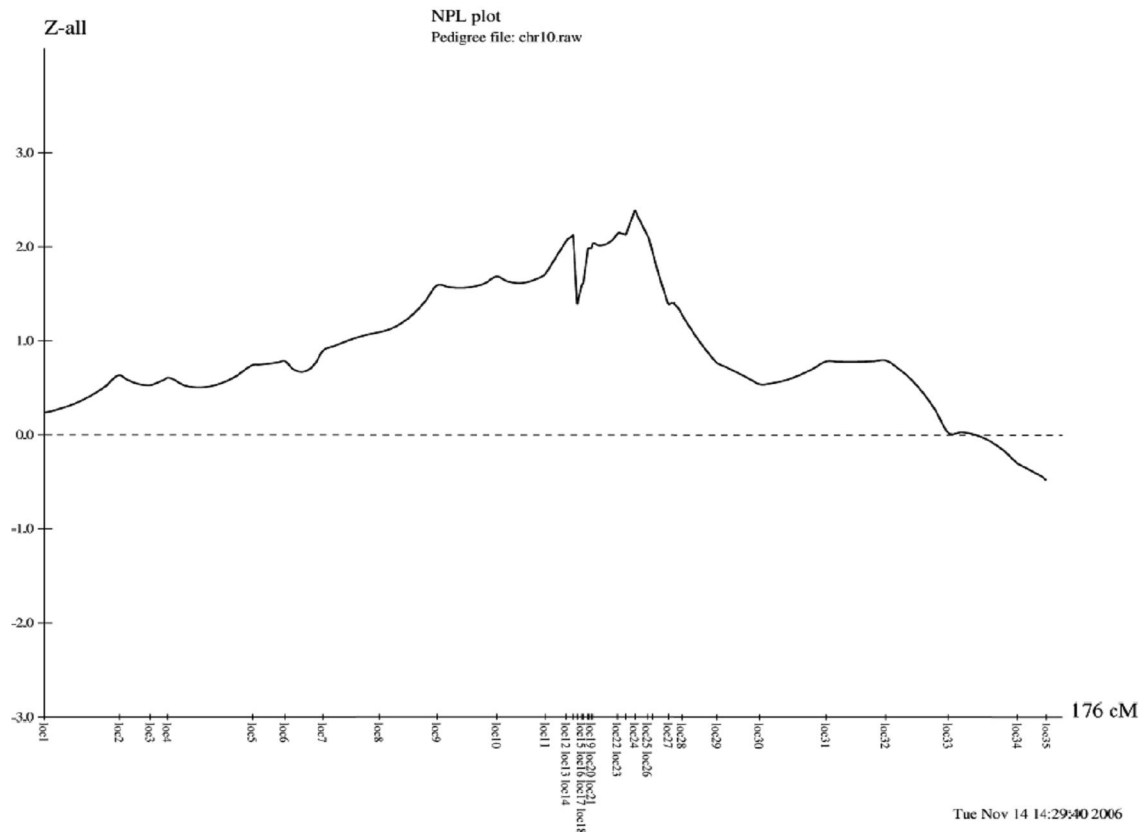
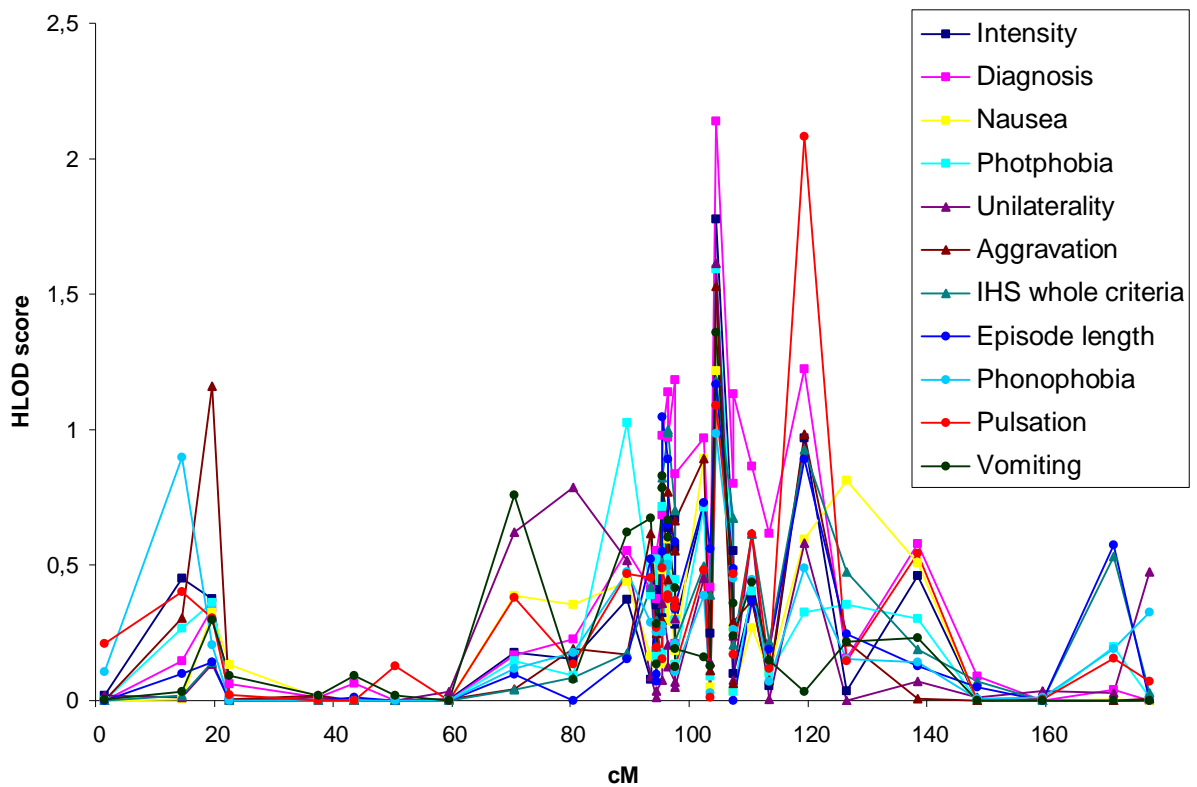


Figure 5. Chromosome 10 parametric linkage analysis



4.1 Discussion

The success rate of the genotyping (90.6 %) was in par with the expected value of about 90 percent.⁸ Some trouble was caused by the single marker D10S2327 in chromosome 10 that had to be omitted from the study. The marker in question produced allele peaks that were low and often virtually impossible to discern from the background stutter. The ineffectiveness of the marker was possibly caused by a bad batch of the specific fluorescent marker used in the capillary electrophoresis. Because it had to be removed from the study, the accuracy of the study fell by a small degree, as the distance between two markers became larger. In total, however, this loss does not hamper the effectiveness of the method, because candidate loci usually correlate to several different markers.

Offset of allele peaks from the binsets was noted as one of the reasons for rejecting data. This phenomenon may have several causes, but a likely reason is the use of too varied genotype data for the creation of the binsets, as suggested by Sarahonka⁹. Offset from the assumed binfiles is, however, comparable to other similar studies conducted in our laboratory. Peaks that were not inside the designated binsets were rejected resulting into a smaller sample size, thus reducing the statistical efficiency of the study. The relative rareness of these cases, however, means that the results of the study are not affected to a large degree. Another problem previously experienced in the laboratory, with the same equipment, was color bleed possibly creating false allele peaks.⁹ This may have caused false allele peak identification, although no evidence suggests that it occurred. Incorrect genotyping, of course, greatly hampers the effectiveness of both linkage and association analysis.

Several different problems with the allele peak sizes and shapes were observed. As the samples underwent PCR before the electrophoresis it is possible that problems in the PCR affected the peak sizes and shapes. Previous studies show that if the PCR process does not work optimally, allele peak sizes are affected. Problems in the PCR process may be due to incorrect temperature during the reaction. As incorrectly sized allele peaks were all rejected, their absence reduced the effectiveness of the study.

Several studies have shown that parametric linkage analysis based LOD scores may not be the best way to map complex characters, such as migraine, because it is difficult to define an

inheritance model and penetration of the disease in the population.⁸ This stems from the fact that complex characters are difficult to identify, or diagnose as in the case of diseases like migraine. Diagnoses are usually based on clinical needs of treatment, whereas the underlying physiological factors in two individuals exhibiting similar complex characters may be different. To counter this problem the parameters used in this study were chosen so they would not significantly affect the results of the analysis.¹⁰ Even the trait component model used in this study is most likely an over simplification of the real inheritance of migraine, although it has already revealed numerous possible migraine loci. Therefore both parametric and non-parametric linkage analysis, are almost always performed in linkage studies. Anttila et al have managed to use parametric linkage analysis to locate migraine loci.² However, because trait component analysis is a new and not fully accepted method in the study of migraine and other complex characters no generalisations can be made of its merits with parametric linkage analysis.

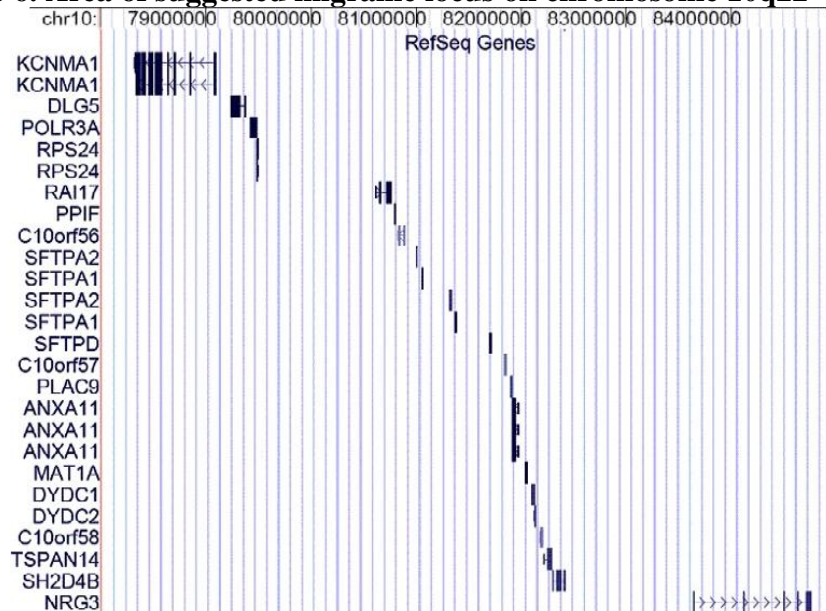
Both parametric and non-parametric multipoint linkage analysis of the results indicated evidence of linkage in chromosome 10q22 around 104 cM. The previously known migraine locus is several cM wide, with numerous different markers showing evidence of linkage, and contains several genes of interest. The evidence of linkage can be considered significant, because many different markers show evidence of it. Anttila et al and Nyholt et al also report the same locus in recent, unpublished, studies in Finnish and Australian populations. Finding the same migraine locus in several geographically and culturally separate populations indicates that the underlying gene linked to migraine is a major component of the disease and also significantly reduces the possibility of the evidence of linkage being falsely positive.

The TDT association analysis showed evidence of association at marker D10S1696. No nearby markers gave any signals of association. Several reasons could be used to explain the results. There is a theoretical possibility that the founder of the migraine population, i.e. the individual who first passed on the mutated candidate gene, lived such a long time ago that recombinations have mixed up the genetic material surrounding the gene so that the individuals sharing the gene do not share much of the surrounding genetic material. Too sparsely spaced markers in chromosome 10q22 may have diluted the results obtained from linkage and association analysis in this study. Results obtained by association analysis are not, however, usually applicable when searching for complex characters, due to the nature of association analysis

algorithms. Therefore the association results obtained in the study are interesting, but cannot be considered to be reliable.

Promising genes close to the markers showing evidence of linkage are KCNMA1 and NRG3 (Figure 6), as seen in the figure 3. KCNMA1 is a calcium activated potassium channel that plays an important part in the depolarization of the plasma membrane of neurons and affects their excitability in synapses.⁶ KCNMA1 has not been previously linked to migraine, but because dysfunctional ion channels are believed to be one of the causes of migraine it is important to study the location further. NRG3 belongs to family of regulatory genes expressed in the neural system, although the exact biological function of NRG3 is yet to be fully discovered. As migraine is a neurological disorder, NRG3 can also be considered as a strong candidate gene for migraine susceptibility. These findings confirm that the locus found in the study is indeed related migraine, but also suggest that the same locus is associated with migraine in various different populations. To determine which gene, and what allele of it, plays a role in migraine susceptibility will be the focus of further studies.

Figure 6. Area of suggested migraine locus on chromosome 10q22¹⁴



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Internet resources

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Acknowledgments

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Appendix 1

Migraine Subtypes According to the International Classification of Headache Disorders, 2nd Edition, 2004¹⁰

- 1.1 Migraine without aura
- 1.2 Migraine with aura
 - 1.2.1 Typical aura with migraine headache
 - 1.2.2 Typical aura with nonmigraine headache
 - 1.2.3 Typical aura without headache
 - 1.2.4 Familial hemiplegic migraine
 - 1.2.5 Sporadic hemiplegic migraine
 - 1.2.6 Basilar-type migraine
- 1.3 Childhood periodic syndromes that are commonly precursors of migraine
 - 1.3.1 Cyclical vomiting
 - 1.3.2 Abdominal migraine
 - 1.3.3 Benign paroxysmal vertigo of childhood
- 1.4 Retinal migraine
- 1.5 Complications of migraine
 - 1.5.1 Chronic migraine
 - 1.5.2 Status migrainosus
 - 1.5.3 Persistent aura without infarction
 - 1.5.4 Migrainous infarction
 - 1.5.5 Migraine-triggered seizure
- 1.6 Probable migraine
 - 1.6.1 Probable migraine without aura
 - 1.6.2 Probable migraine with aura
 - 1.6.3 Probable chronic migraine

Appendix 2.

LOD-scores parametric linkage analysis of chromosome 10

Location (cM)	Marker	Intensity	Diagnosis	Nausea	Photophobia	Unilaterality	Aggravation	IHS whole criteria	Episode length	Phonophobia
1,18	D10S249	0,018067	0,000000	0,000000	0,000000	0,019022	0,000000	0,000000	0,000000	0,106880
14,38	D10S591	0,450667	0,144577	0,004039	0,265441	0,011379	0,305483	0,018762	0,099540	0,897730
19,78	D10S189	0,376099	0,340009	0,325764	0,360986	0,135804	1,160869	0,142101	0,141102	0,204249
22,84	D10S1779	0,000043	0,060497	0,131765	0,000000	0,000000	0,004951	0,000000	0,000000	0,000000
37,75	D10S1653	0,000000	0,011205	0,011031	0,000478	0,011769	0,017024	0,000000	0,000000	0,000000
43,40	D10S548	0,000000	0,064406	0,002389	0,000000	0,000000	0,000000	0,000000	0,009468	0,000000
50,04	D10S197	0,000000	0,000000	0,000000	0,000000	0,000000	0,000000	0,000000	0,000000	0,000000
59,94	D10S208	0,002302	0,000000	0,000000	0,000000	0,033354	0,004604	0,000000	0,000000	0,000000
70,07	D10S196	0,176584	0,166769	0,386522	0,145272	0,621085	0,041388	0,040563	0,094416	0,118215
80,61	D10S1652	0,152872	0,225573	0,355513	0,090550	0,785595	0,190482	0,085903	0,000000	0,177496
89,16	D10S537	0,371105	0,553595	0,439072	1,023154	0,518982	0,168941	0,175021	0,152524	0,475205

93,07	D10S218	0,078086	0,4130140,163251	0,391082	0,090203	0,616351	0,419832	0,522543	0,285983
94,02	D10S535	0,407759	0,3761420,154088	0,163816	0,011987	0,290152	0,199602	0,069617	0,253715
94,72	D10S195	0,352126	0,5540730,133546	0,522152	0,034092	0,246896	0,294452	0,095632	0,252586
95,52	D10S580	0,306395	0,6846650,133155	0,177930	0,075263	0,293540	0,508950	0,548123	0,277601
95,79	D10S202	0,536875	0,9794640,483022	0,717845	0,357164	0,796626	0,821989	1,045955	0,250892
96,62	D10S109	0,522369	1,1393280,292454	0,402982	0,206985	0,445847	0,990626	0,888523	0,502435
97,46	D10S206	0,674894	0,2086780,303181	0,446324	0,301053	0,368282	0,360768	0,337534	0,207332
97,46	D10S569	0,281553	0,8363210,182881	0,132938	0,049162	0,662125	0,219796	0,350432	0,106055
96,83	D10S1730	0,639716	0,9720380,597068	0,525670	0,124773	0,769222	0,998486	0,648575	0,495269
97,22	D10S605	0,564062	1,1832790,134718	0,299359	0,068749	0,554029	0,701429	0,585125	0,208765
102,04	D10S1696	0,718714	0,9695620,893952	0,711157	0,450494	0,895211	0,495183	0,729354	0,386044
103,26	D10S1786	0,247027	0,4170100,048554	0,087423	0,140407	0,109225	0,389866	0,560674	0,027491
107,34	D10S1687	0,551728	0,8027060,270261	0,481285	0,239209	0,063364	0,673156	0,484586	0,451058
104,85	D10S1686	1,775960	2,137554 1,214852	1,590734	1,613057	1,527197	1,181064	1,165560	0,986934
107,92	D10S541	0,100583	1,1329440,031964	0,033093	0,074004	0,291585	0,206637	0,000565	0,261185
110,74	D10S2470	0,378531	0,8640720,268437	0,405892	0,616568	0,368673	0,614874	0,366675	0,448452
113,13	D10S185	0,052897	0,6155260,107140	0,100887	0,002519	0,072918	0,217755	0,187572	0,072223
119,26	D10S192	0,966913	1,2235380,594549	0,325721	0,581694	0,982895	0,926784	0,890608	0,489537
126,70	D10S597	0,034744	0,1558250,811219	0,356035	0,000000	0,215149	0,476074	0,245550	0,151178
138,39	D10S1693	0,461221	0,5763090,508559	0,302356	0,072006	0,006732	0,188180	0,126206	0,142231
148,87	D10S587	0,000000	0,0887700,000391	0,000000	0,012160	0,000000	0,070052	0,049596	0,006124
159,85	D10S217	0,000000	0,0000000,000434	0,000000	0,035873	0,001216	0,000000	0,000000	0,010032
171,95	D10S1651	0,000000	0,0402590,003387	0,199428	0,027317	0,001737	0,533183	0,575440	0,190612
177,19	D10S212	0,000000	0,0000000,000000	0,011465	0,474423	0,005472	0,027491	0,000000	0,326503

Appendix 3. p-values of association of diagnosis phenotype of chromosome 10

Marker	Location(cM)	Diagnosis p-value
D10S537	89,16	0,5197
D10S218	93,07	0,8004
D10S535	94,02	0,5514
D10S195	94,72	0,6618
D10S580	95,52	0,7753
D10S202	95,79	0,7924
D10S109	96,62	0,6555
D10S1730	96,82	0,6580
D10S605	97,22	0,8585
D10S206	97,46	0,8577
D10S569	97,46	0,7370
D10S1696	102,04	0,0017
D10S1786	103,26	0,6168
D10S1687	107,34	0,2124
D10S541	107,92	0,9713
D10S2470	110,74	0,6309